

RNA: Disulphide Bond Containing Oligodeoxyribonucleotide Duplexes Selectivity Studies

Ajay Kumar

Galgotias University

Plot No-2, Sec-17 A, Yamuna Expressway, Greater Noida Budha Distt. Gautam Nagar, UP, India
ak.gupta59@rediffmail.com

Abstract

Selectivity studies of RNA: disulphide bond containing oligodeoxynucleotide duplexes using melting temperature (T_m) have been reported. The T_m data of the RNA/ DNA duplexes showed that duplex formed with disulphide bond containing oligodeoxynucleotide melted at higher temperature than that of RNA/ unmodified DNA duplex by 9.5°C. Even RNA /oligodeoxynucleotide with adjacent thiol groups formed more stable duplex compared to RNA/unmodified DNA duplex. Studies also showed that the selectivity of duplexes with disulphide bond was out performed duplexes with unmodified oligonucleotide.

Keywords

C-5 Thiopropyne Thymidine Substituted Oligonucleotides; T_m Studies; Selectivity; Disulphide Bond Containing Oligonucleotides

Introduction

Synthetic oligonucleotides have greater potential to become a new type of rationally designed therapeutic agent. These compounds could potentially interfere with the expression of selected genes through interaction with mRNA, genome DNA^{1,2} or regulatory proteins³⁻⁷. Antisense oligonucleotides recognize target mRNA sequence through Watson-Crick hydrogen bonding among A and T and G and C. This recognition is highly specific and should lead to development of less toxic and more site specific therapeutic agents⁸. The use of oligonucleotides as antisense inhibitor of gene expression^{1,9} and probes for RNA processes¹⁰⁻¹² requires high affinity for RNA.

The concept used in antisense technology is relatively straightforward. The antisense technology makes use of oligonucleotide sequence, complementary to a specific mRNA, which can inhibit its expression and then a blockade is induced in the transfer of genetic information from DNA to protein. The antisense oligonucleotides are commonly used both in the laboratory and clinic. In general, relatively short

oligonucleotides (13-25 nucleotides) hybridize (at least in theory) to a unique sequence in the total pool of targets present in cells. However, nonspecific effects occur with phenotype which cannot be ignored. The use of phosphodiester oligonucleotides is limited as they are rapidly degraded by the intracellular endonucleases and exonucleases, usually via 3'→5' activity²⁷⁻²⁹. In addition degradation products of phosphodiester oligonucleotides, dNMP oligonucleotides, may be cytotoxic and also exert antiproliferative effects³⁰⁻³³. Deoxyribonucleotide phosphodiester oligonucleotides should therefore not be employed in antisense experiments. The use of oligonucleotides as antisense inhibitor of gene expression^{1,9} and probes for RNA processes³⁴⁻³⁶, requires high affinity and selectivity for RNA. So there are basically two main problems in making use of oligonucleotides as therapeutics—(i) degradation of oligonucleotides (ii) non-specific binding of oligonucleotides to the target mRNA, the first of which has been studied in details by making nucleases resistance phosphodiester bonds and also by other modifications³³, while the later doesn't. The C-5 propyne analogs of 2'- deoxycytidine has been shown to significantly enhance double helix formation with single strand RNA, relative to thymidine and 5-methyl-2-deoxycytidine¹³ to improve the affinity of single strand ligand towards single strand RNA. Various other successful modifications have been carried out^{14-23, 37-41}. Recently we have described, disulphide containing oligonucleotide stabilizes DNA: RNA structure²⁶. Here in this paper, RNA/ disulphide bond containing oligonucleotide duplex formation and selectivity studies have been discussed.

Material and Methods

Synthesis of Oligonucleotides

The unmodified oligonucleotides (deoxy- as well as

ribo-) and C-5 thiopropyne substituted thymidine with oligonucleotide were synthesized, deprotected, purified as described before²⁴ as well as base composition analysis of oligonucleotides. The preparation of disulphide bond containing oligonucleotide and purification has been made²⁵. The duplexes having free thiol groups for studies were obtained by reducing the disulphide bond as described earlier²⁶. A portion of duplex (R+2) was separately treated with 100 mM DTT overnight and dialyzed against water (4X2L) and finally dried thereafter and 500 UL buffer was added. The resulting solution was kept at 4°C overnight.

Thermal Denaturation Studies

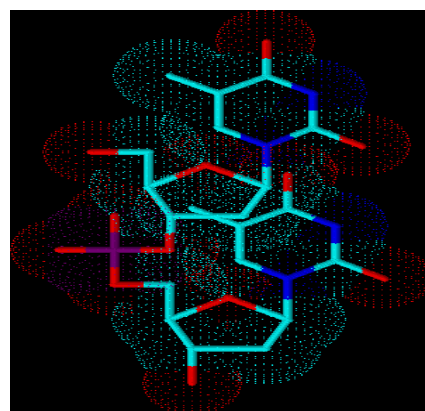
Solutions to the thermal denaturation studies of duplex contained one is to one ratio of a DNA oligomers and complementary RNA target oligomer (1.5 u M each) in a buffer (100 mM, NaCl, 100 mM MgCl₂, 100 mM NaPIPES) at 7 pH were prepared. The mixtures were heated to 90°C, then cooling down slowly to room temperature prior to the melting experiments, carried out in Teflon –Stoppered 1 cm path length quart cells under nitrogen atmosphere on a Varian Carry UV- VIS Spectrophotometer equipped with thermoprogrammer. Absorbance (260 nm) was monitored while temperature was raised from 10°C at a rate of 0.5°C/ min.

Results and Discussion

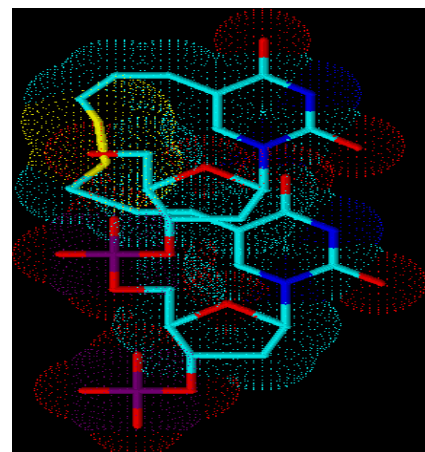
The oligonucleotides sequences synthesized are tabulated in Table-1. *T_m* data of the duplexes determined is tabulated in Table –2. The ball and stick models of three types of dimers are shown in Fig.-1, a-c. In stick model of thymidine dimer, the methyl groups of thymidines are far apart whereas the –SH groups in the dimer of free thiols attached Thymidines, two thiol groups are quit close to each other. The polar surface areas of two thiols are overlap. The stick model of disulphide containing dimer shows that disulphide bond further brings two thiol groups near to each other and restricts the rotation of propyl bonds.

The duplexes are derived from oligonucleotides (1, 2 and 2a) and complementary single stranded RNA as well as mismatch with RNA sequences. *T_m* profiles of the duplexes are shown in Fig- 2, 3, 4. The *T_m* profiles of the duplexes are sharp and *T_m* is easily determined. The data (Table–1) has showed that melting temperatures (*T_m*s) of the duplexes [R (i-iv) +2] with disulphide bonded oligonucleotide were higher than that of the duplexes [R (i-iv) +1] with unmodified oligonucleotide. *T_m* of the duplex (R+2) was higher than that of (R+1) by 9.5°C²⁶. The oligonucleotide (2)

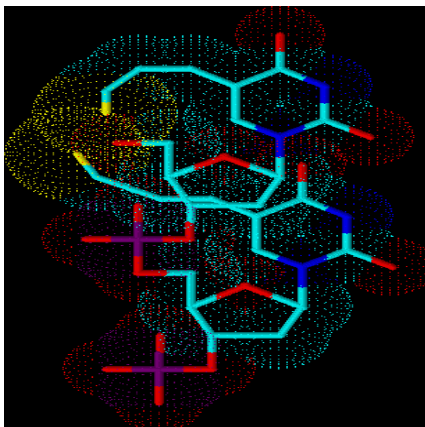
showed selectivity for wobble base having RNA oligonucleotides in the range of 23.5-24.5°C. Whereas the oligonucleotide (1) showed selectivity for wobble base with RNA oligonucleotides in the range of 16-19.0°C. The oligonucleotide (2a) which contains reduced disulphide bond (i.e. two thiol groups) stabilized duplex better than unmodified but relatively less than to the disulphide with oligonucleotide. The selectivity of thiols containing oligonucleotide (2a) with wobble bases was comparable with unmodified oligonucleotides but relatively less than the disulphide including oligonucleotide (2).



a) STICKS STRUCTURE OF THYMIDINES DIMER



b) STICKS STRUCTURE OF DISULPHIDE WITH THYMIDINES



c) STICKS STRUCTURE OF FREE THIOLS ATTACHED TO ADJACENT THYMIDINES

FIG.1 BALL AND STICK MODELS OF DIMERS

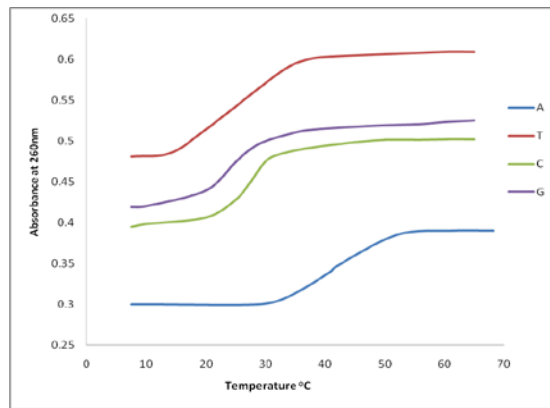


FIG. 2 T_m PROFILES OF UNMODIFIED OLIGODEOXYNUCLEOTIDE: OLIGORIBONUCLEOTIDES DUPLEXES

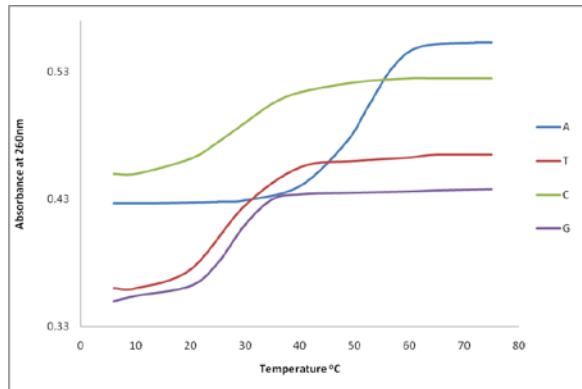


FIG. 3 T_m PROFILES OF DISULPHIDE WITH OLIGODEOXYNUCLEOTID: OLIGORIBONUCLEOTIDES DUPLEXES

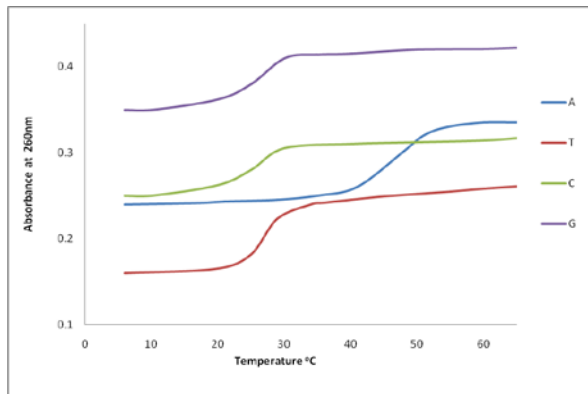


FIG. 4 T_m PROFILES OF REDUCED DISULPHIDE WITH OLIGODEOXYNUCLEOTIDES: OLIGORIBONUCLEOTIDES DUPLEXES

TABLE 1 OLIGONUCLEOTIDES SYNTHESIZED

S.No	Oligonucleotide Sequence
RNA Seq.	3' AAGAAXGAAAAG 5'
	X= T,A,G,C
1	5' TTC TTTCTTTTC 3'
2	S-S II 5' TTCT T TCTTTTC 3'
2a	HS SH II 5' TTCT T TCTTTTC 3'

TABLE 2 T_m DATA OF OLIGONUCLEOTIDE DUPLEXES

T _m o C					
Duplexes	X=	A	T	C	G
R+1 (Fig.-1)		42.5	26.5	24.0	23.5
Selectivity(S)			16.0	18.5	19.0
R+2(Fig.-2)		52.0	28.5	27.5	27.5
(S)			23.5	24.5	24.5
ΔT _m		09.5			
R+2a(Fig.-3)		46.0	28.5	26.0	26.0
(S)			17.5	18.0	18.0
ΔT _m		03.5			

(S) Selectivity is defined as the difference between T_m of the correct oligonucleotide sequence and mismatch oligonucleotides sequences

Conclusion

Our T_m studies results have clearly showed that disulphide with oligonucleotide stabilizes the RNA/ DNA duplex structure significantly more than unmodified oligonucleotide²⁶. The disulphide with oligonucleotide also showed better selectivity for wobble base containing ribooligonucleotides than the unmodified oligonucleotide. The oligonucleotide with reduced disulphide bond (i.e. two free thiol groups) has stabilized RNA/DNA duplex relatively better than duplex with unmodified oligonucleotide. Hence both high stabilization and selectivity of RNA/ DNA duplexes are due to the presence of disulphide bond which stabilizes the conformation as shown in Fig-1. This stabilization and improved selectivity may result from the favorable geometry of disulphide bond present in the center of the oligonucleotide. The oligodeoxynucleotide, 2a which contains reduced disulphide bond (i.e. two thiol groups) stabilized duplex better than unmodified but less than disulphide bond containing oligonucleotide. Earlier Glick *et al.*⁴²⁻⁴³ have reported conformationally restricted DNA hairpin with disulphide cross link increasing the T_m by 21°C relative to the wild type sequence. Froehler *et al.*¹³ have used oligodeoxynucleotides with C-5 propyne analogs of 2-deoxyuridine and 2-deoxycytidine for stabilization of duplexes. The stabilization of duplex in case of cross linked oligonucleotide is due to the restriction in flexibility and in case of oligodeoxynucleotides containing C-5 propyne analogs of 2-deoxyuridine and 2-deoxycytidine is due to π- π* interactions. The thiopropyne modified oligonucleotide contains triple bonds to promote π- π* interactions as well as a disulphide bond between the two adjacent deoxyuridines to restrict the conformation.

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